Production of Rutin from Buckwheat*, †

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Methods for the extraction and purification of rutin from either green or dried buckwheat meal are described. The best solvent and extraction time for optimum yields of rutin were determined by comparing and evaluating various solvents. For the green plant ten minutes extraction time with hot 85 per cent isopropyl alcohol is recommended. For buckwheat meal 70 to 85 per cent isopropyl alcohol, either hot or cold, is the most efficient extraction solvent for rutin.

RUTIN IN quantity for clinical use was first produced from flue-cured tobacco in 1942. However, as mentioned in a previous publication (1) it had previously been identified in tobacco by other investigators. In our laboratory it was prepared by percolation with ethyl alcohol, a procedure similar to that used by Sando and Lloyd (10) for the extraction of elder flowers. The low yields from an expensive raw material led to a search for a more economical source of the glucoside. This resulted in the selection of buckwheat (5) as the most promising of the various plants investigated. As previously mentioned (5) rutin was first isolated from buckwheat by Schunck in 1860. As a matter of historical record, rutin from buckwheat was first prepared in quantity from the fresh green plant in June, 1944, by a technique similar to that used for ue-cured tobacco. Several articles have since een published on production of rutin from buckwheat (3, 4, 8) for the purpose of giving information to manufacturers. The purpose of this paper is to describe the methods used for solvent extraction of buckwheat and to present some of the data on which manufacturing recommendations have been based. The extraction procedures have been changed from time to time as more knowledge of the chemical and physical properties of rutin became available.

At present, the most widely used method for preparing rutin employs hot isopropyl alcohol extraction of either fresh green buckwheat or a leaf meal obtained by drying the green plant. Optimum conditions for production of the leaf meal have been described (8).

Tartary buckwheat has been used for most of the experiments reported here because data obtained over three growing seasons (6) have demonstrated the superiority of this variety over other common varieties.

* Received October 27, 1949 from the Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia 18, Pa.

† Presented in part at the Meeting-in-Miniature of the hiladelphia Section of the American Chemical Society, anuary 20, 1949.

‡ The authors thank J. Naghski for timely suggestions; R. W. Bruch for technical assistance; J. Naghski and Charles enske for rutin analyses; and W. L. Porter and M. L. Swain spectrophotometric data.

EXPERIMENTAL

Four methods for production of rutin will be presented in this paper. They are (*a*) cold solvent extraction of green Tartary buckwheat; (*b*) cold solvent extraction of commercial Japanese buckwheat whole meal, (*c*) hot solvent extraction of green Tartary buckwheat, and (*d*) hot solvent extraction of Tartary buckwheat leaf meal.

Because of many variables involved in the extraction and the pressing need for an adequate supply of rutin for clinical investigations, few significant data on factors influencing yield of rutin were obtained from the first fourteen preparations, in which from 50 to 500 pounds of fresh plant were processed. Results obtained from these experiments together with solubility data (11) indicated, however, that the strength of the solvent was an important factor influencing yield. The experiments under (*a*) were made to evaluate the efficacy of different strengths of ethyl alcohol in extracting rutin from the green plant.

Cold Solvent Extraction of Green Tartary Buckwheat

Procedure.—Into a 100-gallon stainless steel tank were placed 100 pounds of freshly harvested green buckwheat plant (minus roots). Previous experiments indicated that cutting or chopping the plant does not give more efficient extraction; it serves only to facilitate packing the plant into a tank. A 1-inch layer of absorbent cotton covered with a 4-inch layer of excelsior placed over the valve of the tank previous to addition of the buckwheat served to strain off the dirt. The buckwheat was covered with 50 gallons of ethyl alcohol (75% by volume), which was circulated at the rate of about 6 gallons per minute for four hours. The buckwheat was then allowed to macerate overnight. The first extract (45.6 gallons) was pumped to the evaporator through a small stainless steel filter press equipped with 1.5 square feet of heavy filter sheets (Republic Seitz Sheet No. K-5 was used[1]) backed with canvas. The marc was covered with 50 gallons of ethyl alcohol (75% by volume), and the process used in the first extraction was repeated.

The first extract was evaporated at atmospheric pressure to 12 gallons and held overnight before the addition of the second extract and washings. After twenty hours the second extract (47.6 gallons) was withdrawn and filtered. The marc was washed twice with 10 gallons of 75% ethyl alcohol; the alcohol was circulated for fifteen minutes each

[1] The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

time. These washings and an additional 2 gallons used to wash out the filter press were pumped to the evaporator with the second extract.

The combined extracts and washings were evaporated to 6 gallons. The concentrate was strained through glass wool (2–4) to remove fats[2] and pumped into a stainless steel, steam-heated holding tank containing 6 gallons of boiling water. After boiling for ten minutes to facilitate agglomeration of fats, the concentrate was filtered through 4 square feet of heavy filter sheets (K-5) backed with canvas, after which the filter press was washed with 2 gallons of boiling water.

The filtered solution was allowed to stand in a stainless steel tank overnight for rutin to crystallize The rutin was then filtered off on heavy canvas, slurried in a small quantity of cold water, and refiltered. The slurrying was repeated. The crude rutin was dried at 110° to constant weight. Analysis of the air-dried marc showed no rutin.

This experiment was repeated with menstrua of different alcoholic strengths. Table I contains a summary of the data obtained.

Discussion.—In general, yields of crude rutin, were low when cold alcohol was used. The reason is not definitely known, but it may be due to the failure of cold alcohol to penetrate the cell wall of the plant in sufficient quantity to inhibit enzymatic destruction of rutin. The data on ethyl alcohol in the extracts (Table I) lends some support to this idea. From the alcohol strength of the extracts, it is evident that the solvent does not reach equilibrium with the water in the plant cells in the extraction period of twenty hours. The enzyme system of the buckwheat plant is now under investigation in this laboratory.

The best yield of rutin was obtained when cold alcohol of 75% strength by volume was used. This is in close agreement with solubility data for rutin-alcohol-water mixtures. Previous experiments, as well as subsequent experiments showed the impracticability of extracting buckwheat with solvent strength as low as 65% by volume. This was due to formation of fat emulsions on evaporation of the extracts, which seriously interfered with precipitation of rutin.

The data indicate also a slight gain in yield of rutin with separate working-up of each extract as obtained. This is shown by comparison of Experiment 166-164 with 166-148. A slight superiority of cold isopropyl alcohol over cold ethyl alcohol as an extractant is indicated by comparison of Experiment 166-171 with 166-148. After suitable methods for the preparation of a leaf meal with good rutin content had been developed, (8), studies were made on extraction procedures for buckwheat meal. These together with those on extraction of the green plant led to the selection of isopropyl alcohol for use in further studies. In addition to being more efficient with respect to yield of rutin especially when used in hot extraction, it has the added advantage of lower cost compared to the denatured alcohols and it is less expensive because of its lower heat of vaporization, which results in power conservation. Moreover, isopropyl alcohol is free of Internal Revenue restrictions.

Cold-Solvent Extraction of Commercial Japanese Buckwheat Whole Meal

In previous cold-solvent extractions of buckwheat meal, approximately 70% alcohol was indicated by solubility data as the most promising solvent. Typical of the percolation data are those of Experiment 166-18 (Tables II and III), in which twenty-hour extracts were made until the marc was free of rutin. In this experiment two 100-gallon stainless steel tanks were packed with buckwheat meal. The meal in the first tank (193.5 pounds or 87.8 Kg.) was covered with 80 gallons of 70% ethyl alcohol and allowed to macerate overnight. The first extract of 49 gallons was withdrawn and worked up for crude rutin. New solvent was added to cover the marc, which was again allowed to stand overnight. This extract was withdrawn and used, together with sufficient additional 70% alcohol to make 80 gallons, to cover the 175 pounds (79.4 Kg.) of meal in the second tank. Fresh solvent was again used to cover the marc in the first tank. After overnight maceration the extract was withdrawn

[2] The chemical composition of these fats, a by-product in the rutin manufacturing industry, will be the subject of another paper.

TABLE I.—EFFICIENCY OF ETHYL ALCOHOL AT VARIOUS STRENGTHS IN COLD-SOLVENT EXTRACTION OF GREEN TARTARY BUCKWHEAT[a]

Experiment No.	Ethyl Alcohol Strength (by Vol.), %	Ethyl Alcohol in Extracts, % Extr. 1	Extr. 2	Analysis of Plant, % Moisture	Crude Rutin, M.F.B.	Rutin in 45.4 Kg. of Plant (Based on Crude Rutin Analytical Value), Gm.	Yield of Crude Rutin Based on Analytical Value Gm.	%
166-128	75	65	74	85.5	2.97	199.4	120.9	60.6
166-134	80	73	78	83.6	2.58	191.9	103.9	54.1
166-140	85	81	85	88.5	2.88	192.1	94.1	48.9
166-142	90	80	88	84.8	2.97	204.6	89.4	43.7
166-148	95	90	95	90.5	3.87	166.7	86.3	51.8
166-164[b]	95	..	...	91.3	3.84	151.6	82.9	54.7
166-144	100	90	100	85.9	2.68	171.4	87.5	51.1
166-150	100	90	100	82.9	3.39	269.0	140.0	52.0
166-171	98–99[c]	..	...	87.9	3.55	194.8	105.9	54.4

[a] 45.4 Kg., 100 lb., was extracted twice for twenty hours each with 50-gallon portions of solvent followed by two 10-gallon washes.
[b] Extracts were worked up separately for crude rutin.
[c] Isopropyl alcohol.

TABLE II.—EXPERIMENT 166-18: EXTRACTION OF RUTIN FROM JAPANESE BUCKWHEAT MEAL BY PERCOLATION WITH 70% ETHYL ALCOHOL

Extract No.	Ethanol Used to Cover, Gal.	Ethanol Withdrawn, Gal.	Extract Concentrated to, Gal.	Yield of Crude Rutin, Gm.	Portion of Total Crude Rutin, %
1	80	49	4	1100.0	20.4
2	80	28	6	947.0	17.5
3	50	29	5	830.0	15.4
4	30	46	4	915.0	17.0
5	17	17	4	355.0	6.6
6	32	32	5	267.0	4.9
7	34	34	5	325.0	6.0
8	32	32	4	250.0	4.6
9	35	35	3	188.7	3.5
10	25	25	2	105.0	1.9
11	37	37	2	69.0	1.3
12	37	37	2	41.0	0.8
13	30	30	2	2.5	> 0.1
14	40	40			
	Total 559	471		5395.2	

TABLE III.—EXTRACTION EFFICIENCY OF VARIOUS SOLVENTS IN COLD SOLVENT EXTRACTION OF JAPANESE BUCKWHEAT MEAL

Experiment No.	Solvent in Extraction	Total Quantity of Solvent, Gal.	Weight of Meal, Kg.	Analysis of Meal, %		Rutin in Meal (Based on Crude Rutin Anal. Value), Gm.	Yield of Crude Rutin Based on Crude Rutin, Analytical Value		Yield of Refined Rutin, Based on Crude Rutin Analytical Value	
				Moisture	Crude Rutin M.F.B.		Gm.	%	Gm.	%
166-18[a]	70% ethyl alcohol	559	167.2	9.00	2.61	3970.1	5395.2	135.9	2955.2	74.4
166-42[a]	70% ethyl alcohol	255[b]	45.7	6.53	2.47	1055.8	1210.0	114.6	802.4	76.0
166-46[a]	70% ethyl alcohol	270[c]	39.9	6.85	2.69	999.4	1114.1	111.5	764.4	76.5
166-55[d]	70% ethyl alcohol	40[e]	4.54	6.53	2.66	112.8	120.3[f]	106.6	97.9	86.9
166-57[d]	70% isopropyl alcohol	40[e]	4.54	6.53	2.66	112.8	116.7	103.5	99.4	88.1
166-59[d]	70% methyl alcohol	40[e]	4.54	6.53	2.66	112.8	105.6	93.6	86.8	77.0
66-82[d]	70% acetone	0.7[g]	0.11	8.60	3.08	3.1	3.4	109.7	2.52	81.8

[a] Rutin was refined by recrystallization from boiling water (3, 5), pharmaceutical quality.
[b] Only 4.0 Gm. rutin was extracted by an additional 70-gallon, twenty-hour extraction.
[c] No rutin obtained by an additional 70-gallon, twenty-hour extraction.
[d] Rutin refined by recrystallization from 10% isopropyl alcohol (3, 5), not pharmaceutical quality.
[e] One 30-gallon, twenty-hour extraction plus two 5-gallon washings. No rutin was found in marcs.
[f] Only 2.6 Gm. of additional rutin was obtained by another 10-gallon, twenty-hour extraction.
[g] One 0.7-gallon, twenty-hour extraction plus two 0.2-gallon washings. No rutin was found in marc.

from the second tank to be worked up for crude rutin. This process was repeated until an extract gave no appreciable rutin on evaporation. Table II shows the crude rutin yield by extracts. Crude and refined yields are recorded in Table III for comparative purposes. The comparative data show that all the available rutin in the meal is extracted by this percolation technique. This procedure offers a convenient method when countercurrent extraction is desirable, and when either quantity of solvent available and (or) size of evaporator are limiting factors in production of rutin from buckwheat meal. Extracts may be withdrawn from percolators in convenient quantities and worked up for crude rutin at suitable intervals.

With a higher solvent-to-plant ratio, it was possible to recover the rutin with fewer extractions. The following procedure was used when only three extractions were made (Experiments 166-42 and 166-46, Table III).

Procedure.—Into a 100-gallon stainless steel tank[3] was placed a false bottom consisting of a 4- to 6-inch layer of excelsior, over which a layer of cheesecloth was spread and tucked in around the edges. The false bottom was anchored with dry meal before addition of solvent. Buckwheat meal (total, 39.9 Kg.) and 70% ethyl alcohol (115 gallons) were then added until the tank was filled. The solvent was continuously recirculated from the bottom to top of the tank at the rate of about 1 gallon per minute for twenty hours. This period was longer than necessary but was used as a convenient day-to-day working period. This action pumps the unscreened fines to the top of the tank and makes a filter bed of the meal, so that the extract soon becomes clear and filtration of the extract before it is transferred to the evaporator is unnecessary. After twenty hours the extract (approximately 85 gallons) was drawn off and evaporated to 8 to 10 gallons at atmospheric pressure. The boiling concentrate was strained through glass wool and then filtered through heavy paper sheets (K-5). The filtrate and washings were allowed to stand for twenty hours for rutin to crystallize. The crude product was filtered off on heavy canvas, slurried with cold water, refiltered, and washed with small portions of cold water. The dry weight of the

[3] The tank used is rated at 100-gallon capacity but actually holds 120 gallons.

TABLE IV.—EXTRACTION EFFICIENCY OF VARIOUS STRENGTHS OF SOLVENT AND EFFECT OF BOILING TIME YIELD OF RUTIN IN HOT ISOPROPYL ALCOHOL EXTRACTION OF GREEN TARTARY BUCKWHEAT[a]

Experiment No.	Solvent Strength (by Vol.), %	Analysis of Plant, % Moisture	Corrected Rutin, M. F. B.	Rutin in 22.7 Kg. (Based on Corrected Rutin Analytical Value), Gm.	Yield of Corrected Rutin Gm.	%
194-121	65	83.3	4.12	156.0	102.5	65.7
194-120	75	87.2	5.40	156.8	119.9	76.5
166-173	85	88.8	3.93	99.8	81.7	81.8
166-175	85	88.2	3.58	95.8	79.7	83.2
166-185	85	86.0	4.16	132.1	108.5	82.1
194-124[b]	85	88.9	5.00	125.9	94.9	75.4
194-122[c]	85	89.9	5.19	118.9	85.5	71.9
166-192	95	82.4	3.06	122.2	84.6	69.2
166-190	98–99	82.4	3.09	123.3	79.7	64.6

[a] 22.7 Kg. (50 pounds) green buckwheat was extracted 3 times for ten-minute periods with 20, 12, and 12 gallons, respectively, of solvent.
[b] First extraction period was sixty minutes.
[c] First extraction period was one hundred and twenty minutes.

TABLE V.—EFFICIENCY OF VARIOUS SOLVENTS IN HOT-SOLVENT EXTRACTION OF GREEN TARTARY BUCKWHEAT[a]

Experiment No.	Solvent	Analysis of Plant, % Moisture	Corrected Rutin, M. F. B.	Rutin in 22.7 Kg. (Based on Corrected Rutin Analytical Value), Gm.	Yield of Corrected Rutin Gm.	%
166-175	85% isopropyl alcohol	88.2	3.58	95.8	79.7	83.2
194-128	85% ethyl alcohol	84.1	3.85	138.8	105.0	75.6
194-125	85% methyl alcohol	86.4	4.54	140.0	83.7	59.8
194-129	75% methyl alcohol	86.2	4.76	149.0	73.0	49.0[b]
194-130[c]	85% acetone	83.0	3.97	3.37	3.10	92.0

[a] 22.7 Kg. (50 pounds) green plant was extracted three times for ten-minute periods, with 20, 12, and 12 gallons, respectively, of boiling solvent.
[b] Analysis of marc showed 21.7% additional rutin.
[c] One-half Kg. sample was used.

crude rutin obtained was 795.0 Gm. or 71.4% of the total crude rutin obtained from all extracts (Experiment 166-46, Table III). The marc was extracted two more times for twenty hours each with 70 gallons of 70% ethyl alcohol. Each of these extracts was evaporated to approximately 6 gallons and handled in the same way as the first extract. From the second extract was obtained 216.0 Gm. or 19.4% of the total weight of crude rutin; from the third extract, 103.1 Gm. or 9.2% by weight of the total crude rutin. A fourth extract yielded no rutin.

Discussion.—In Experiments 166-55, 166-57, 166-59, and 166-82 of Table III, which illustrate the use of still higher solvent-to-meal ratios, good results were obtained by one extraction followed by several washings to remove entrained solvent-bearing rutin.

Attempts to extract buckwheat meal with strong cold acetone have been unsuccessful, and this experience, at an early date in the rutin program, discouraged further work with this solvent. However, if the meal were first extracted with a fat solvent, such as benzene, the rutin might be extracted with strong acetone. Extraction with 70% acetone on a small scale (Experiment 166-82, Table III) gave a satisfactory yield. Further investigation with this solvent is in progress.

No corrected rutin yields are given here because the data were obtained before the variability of benzene soluble and alcohol-insoluble impurities in crude rutin was realized. However, data on the yield of "refined" rutin are included. The values for Experiments 166-18, 166-42, and 166-46 were obtained by purifying the manufactured rutin by crystallization from boiling water, which produced rutin of pharmaceutical quality; those for Experiments 166-55, 166-57, 166-59, and 166-82 were obtained by crystallization from 10% isopropyl alcohol (rutin not of pharmaceutical quality).

Hot-Solvent Extraction of Green Tartary Buckwheat

Several preliminary experiments were made on hot extraction of green buckwheat with strong ethyl alcohol (95% by volume). In one, 50 pounds of green plant were extracted with 20, 12, and 12 gallons[4] of boiling alcohol for ten-minute periods. The yield of crude rutin was 79.4%, as compared with 54.7% for cold alcohol (Experiment 166-164, Table I). When the boiling periods were increased to sixty minutes each, the yield was 62.3%. Use of 85% isopropanol for thirty-minute boiling periods gave a crude rutin yield of 69.3%. These preliminary tests indicated that hot extraction gives a higher yield than cold extraction and that possibly prolonged contact of hot solvent with green buckwheat may result in lower yields.

Table IV summarizes the data of experiments planned to determine the optimum solvent strength

[4] A fourth extraction was not necessary because only 0.7 Gm. of rutin was obtained in an additional 12-gallon extraction.

nd heating time for extraction of green Tartary ıckwheat with isopropyl alcohol. Table V sum-..larizes the data on the efficiency of several of the most promising solvents.

At an early date evidence indicated that crude rutin obtained both in the manufacturing process and by analysis[6] contained two main types of impurities in different amounts—"benzene solubles" and "alcohol insolubles." In these experiments the benzene solubles and alcohol insolubles in the manufactured rutin ranged from 0.05–3.33% and 1.22–7.79%, respectively, whereas in the analytical control samples the range was 0.81–6.33% and 3.58–13.7%, respectively. Since these impurities showed such wide variations, it became essential to correct all rutin values before an evaluation could be made of the factors affecting yields. Rutin freed of these impurities is hereafter referred to as "corrected."

The following extraction procedure is typical of that used in the experiments presented in Tables IV and V.

Procedure.—To a 50-gallon stainless steel, steam-heated kettle were added 50 pounds of whole freshly harvested buckwheat (minus roots). Sufficient isopropyl alcohol (85% by volume) was added to cover the plant (20 gallons). The alcohol was brought to a boil as rapidly as possible (six minutes) and held at boiling temperature for ten minutes. The first hot extract was drawn off, filtered by pressure through 2 square feet of heavy filter sheets (K-5) backed with canvas, and pumped into an evaporator. Similarly, a second and a third ten-minute extract were made; 12 gallons of 85% isopropyl alcohol was enough to cover the marc in each case. Each extract was worked up separately for crude ıtin.

The first extract, approximately 16 gallons, was evaporated at atmospheric pressure to 2.5 to 3 gallons. The boiling concentrate was strained through glass wool and filtered through about 1 square foot of heavy filter sheet. The evaporator and filter were washed with 0.5 gallon of boiling water, and the filtrate and washings were immediately cooled by the addition of 10 to 15 pounds of crushed ice until the temperature was about 5°. After cooling, the mixture containing crude crystallized rutin was allowed to stand at low temperature for one hour, after which it was filtered off on hard-surfaced paper, thoroughly washed with small portions of cold water, and dried at 110° to the constant weight of 64.5 Gm. No further precipitation of rutin occurred after the mother liquors had stood for forty-eight hour periods in a cold room (temperature about 5°). The second and third extracts of approximately 12 gallons each were evaporated to about 0.75 gallon, strained, filtered, and cooled with about 5 pounds of crushed ice. These solutions were allowed to stand for one hour for complete crystallization of crude rutin. The dried crude rutin from the second extract weighed 12.0 Gm., and that from the third extract, 6.5 Gm. Previous experience had shown that a fourth extraction was unnecessary. The entire experiment, from the cutting of the green buckwheat, was completed in a single eight-hour working day.

Discussion.—The data in Table IV show that 85% isopropyl alcohol and a ten-minute boiling extraction period gave the maximum yield of rutin. Heating for longer periods, as in Experiments 194-124 and 194-122, lowered the yield of rutin. An extraction with 55% alcohol (not shown in the table) was highly unsatisfactory because the fats emulsified when the extracts were evaporated. Large amounts of salt had to be used to agglomerate the fat, and then completeness of crystallization was not certain even after forty-eight hours at low temperature. With low alcohol strength, evaporation of solvent was also slow and impracticable, especially at atmospheric pressure.

A breakdown of yields of crude rutin in Experiment 166-175, which is representative of those obtained in other experiments with hot-solvent extraction of green buckwheat, showed that 77.7, 14.5 and 7.8% of the total weight of crude rutin was present in the first, second, and third extract, respectively. It would appear that the last two extractions serve only to wash out rutin-bearing solution from the marc. It seems reasonably safe to assume that complete extraction occurs with one ten-minute hot extraction. It has been suggested that in commercial operations, if batch extraction methods are used, possibly no more than two extractions are needed, one of which might be a thorough hot washing. When a third extraction appears economically feasible, this extract could be used for the first extraction of a new batch.

Data in Table V indicate the superiority of 85% isopropyl alcohol over ethyl and methyl alcohols for the extraction of green buckwheat. The possibilities of dilute acetone have not been thoroughly investigated, but in a preliminary experiment in which a 0.5-Kg. sample was used (Experiment 194-130) 85% hot acetone appeared promising as a solvent for green buckwheat.

Hot-Solvent Extraction of Tartary Buckwheat Leaf Meal

Table VI shows the data for the optimum conditions with regard to the strength of hot solvent and length of heating time for extraction of buckwheat meal. In a preliminary experiment (not included in the table) in which hot 70% methyl alcohol was used as a solvent, the corrected yield of rutin was 60.7%, only about two-thirds that obtained with isopropyl alcohol under similar conditions. The technique used in Experiment 194-9 which is typical of that recommended for the extraction of leaf meal, was as follows.

Procedure.—A false bottom was placed in a 50-gallon stainless steel, steam-heated kettle, and 5 pounds of meal were put in to serve as an anchor. Fifteen gallons of 75% isopropyl alcohol were added, followed by an additional 5 pounds of meal. The alcohol required seven minutes to reach boiling temperature, and the mixture was kept at this point for ten minutes. The first extract was pumped into the evaporator through 2 square feet of heavy paper sheets (K-5) backed with canvas. One gallon of 75% isopropyl alcohol was used to wash the filter press free of the first extract solution. The 12 gallons (approximate) of extract obtained were evaporated at atmospheric pressure to 2 gallons. Three

[6] The method used in these analyses, together with additional data showing the need for correction of the yield of crude rutin has been published since the preparation of this manuscript. Naghski, J., Fenske, C. S., Jr., Krewson, C. F., and Couch, J. F., "Determination of Rutin in Plant Materials," *U. S. Dept. Agr., Bur. Agr. and Ind. Chem.*, AIC-236 (Eastern Regional Research Laboratory), August, 1949. (Processed.)

TABLE VI.—EXTRACTION EFFICIENCY OF VARIOUS STRENGTHS OF SOLVENT AND EFFECT OF BOILING TI ON YIELD OF RUTIN IN HOT ISOPROPYL ALCOHOL EXTRACTION OF TARTARY BUCKWHEAT LEAF MEAI

Experiment No.	Solvent Strength (by Vol.), %	Yield of Crude Rutin, Based on Crude Rutin Analytical Value Gm.	%	Benzene Solubles in Crude Rutin, %	Alcohol Insolubles in Crude Rutin, %	Corrected Yield of Rutin Gm.	%	Crude Rutin in Marc, by Analysis Gm.	%
194-19	55	185.3	97.6	16.92	12.03	131.7	74.3	3.18	1.79
194-17	65	183.5	96.7	0.51	8.49	167.0	94.2	2.84	1.60
194-29	70	187.0	98.5	0.13	6.72	174.2	98.3	1.65	0.93
194-5[b]	75	180.1	94.9	0.09	5.46	170.1	95.9	5.52	3.11
194-9	75	185.4	97.7	0.09	5.19	175.6	99.0	6.90[c]	3.90
194-13[d]	75	177.0	93.3	0.06	4.63	168.7	95.2	None found	None found
194-15	85	175.8	92.6	0.09	0.98	175.7	99.1	8.70	4.91
194-27	90	132.3	69.7	0.08	0.45	131.6	74.2	43.9	24.8
194-25	98–99	41.9	22.1	0.29	3.04	40.5	22.8	137.2	77.4

[a] 4.54 Kg. (10 pounds) of meal was extracted three times for ten-minute periods with 15, 6, and 6 gallons, respectively, of boiling solvent. Meal analyzed 8.10% moisture, 4.55% crude rutin. Rutin contained 5.16% benzene solubles, 0.36% alcohol insolubles. Corrected yield of rutin is 4.30%.
[b] Ten gallons of solvent instead of 15 gallons was used for first extract.
[c] Obtained by two additional 6-gallon extractions.
[d] First extraction period was one hundred and twenty minutes.

gallons of boiling water were added, and boiling was continued for fifteen minutes. An additional gallon of boiling water was added, and the boiling concentrate was strained through glass wool and filtered through heavy filter sheets; about 2 square feet of filtering area were used. The equipment was washed out with a small quantity of boiling water. To the hot filtrate and washings (about 5½ gallons) were added 20 pounds of crushed ice. After one hour, the crude rutin was filtered off on hard-surfaced paper, washed three times with 0.25-gallon portions of cold water and dried at 110° to a constant weight of 138.0 Gm.

A second extract was made with 6 gallons of 75% isopropyl alcohol (by boiling for ten minutes) immediately after removal of the first extract. This second extract was evaporated to 1 gallon, diluted with 2 gallons of boiling water, boiled for fifteen minutes, strained, and filtered. To the rutin filtrate (approximately 3 gallons) were added 10 pounds of crushed ice for rapid cooling. After one hour the rutin was filtered off, washed and dried at 110° to a constant weight of 37.0 Gm.

A third extract was made by the same technique as that described for the second extract. However, since this extract contained less rutin, it was evaporated to 0.5 gallon instead of 1 gallon, in order to insure complete crystallization of rutin in one hour. The yield of crude rutin from the third extract was 10.4 Gm., making the total yield from the three extracts 185.4 Gm. Since a total yield of only 5.9 Gm. was obtained from two additional 6-gallon extracts, just three extracts were made in all subsequent experiments. Data for these experiments are included in Table VI. The entire procedure—making the three extractions and placing the crude rutin in the oven to dry—required one working day.

Discussion.—Again the necessity for correcting the figure for yield of crude rutin of both manufactured and analytically determined rutin for benzene solubles and alcohol insolubles is demonstrated by the data in Table VI. Reasonable checks can be obtained in the extraction of buckwheat meal with hot solvent as demonstrated in the extraction of green plant with hot solvent, provided corrected rutin values are compared.

From the quantity of benzene solubles and alcohol insolubles in the crude rutin, together with corrected yield figures in Table VI, it appears that 85% is the optimum strength to be recommended, although any concentration from 70% to 85% is practical. With alcohol of lower strength, fat emulsion difficulties are encountered in evaporation of solvent, and large amounts of electrolyte must be added to break such emulsions. Even so, it will be noticed from Experiments 194-19 and 194-17 that with 55% and 65% isopropyl alcohol extraction the benzene solubles and alcohol insolubles in the crude rutin are increased with decrease in solvent strength. This means that greater difficulty will be encountered in refining to a pharmaceutically acceptable product. Above 85% strength, the yield of rutin sharply diminishes, which is in close agreement with solubil data for rutin in boiling isopropyl alcohol-water mi tures. In extraction of buckwheat meal, heating for

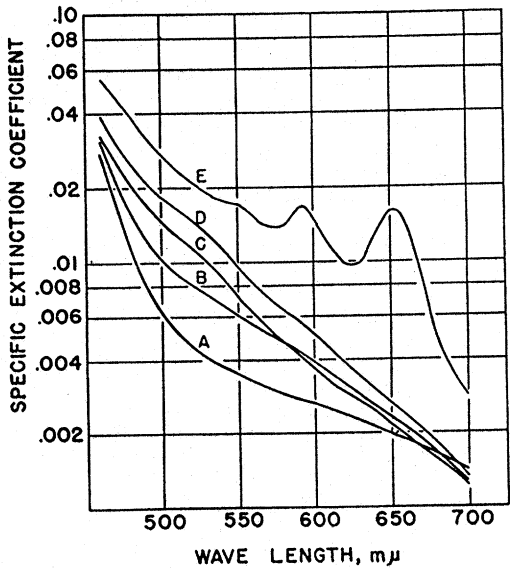

Fig. 1.—Spectrophotometric curves. *A*, highly purified rutin; *B*, sample 166-179; *C*, sample 16b 46; *D*, sample 194-9; *E*, crude rutin. Curves fo other samples in Table VII fall within limits bounded by *B*, *C*, and *D*.

TABLE VII.—PURITY OF SAMPLES OF RUTIN PREPARED BY VARIOUS METHODS OF EXTRACTION[a]

Experiment No.	Crude Rutin Prepared by—	Rutin after Refining, Gm.	Rutin,[b] %	Quercetin,[b] %
166-175	Hot 85% isopropyl alcohol extraction of green buckwheat	7.66	98.5	0.8
166-179	Hot 75% isopropyl alcohol extraction of green buckwheat	7.32	97.4	0.2
166-140	Cold 85% ethyl alcohol extraction of green buckwheat	7.73	99.2	1.6
166-128	Cold 75% ethyl alcohol extraction of green buckwheat	7.33	98.4	1.6
194-15	Hot 85% isopropyl alcohol extraction of leaf meal	7.83	97.8	1.0
194-9	Hot 75% isopropyl alcohol extraction of leaf meal	7.33	97.4	1.4
166-46	Cold 70% ethyl alcohol extraction of whole meal	764.5[c]	96.1	2.2

[a] Purified by recrystallization of 10.0 Gm. of crude rutin from boiling water; 5.0 Gm. of silica gel was used for removal of red pigment. Not more than a trace of absolute ethanol insoluble impurities was present.
[b] Rutin, quercetin, red pigment, and chlorophyll were determined by the method of Porter, Brice, Copley, and Couch (9). Red pigment in each sample was less than 0.0001%; chlorophyll was less than 0.001%.
[c] From recrystallization of 1114 Gm. of crude rutin.

more than ten minutes was not so deleterious to rutin yields as it was in extraction of green buckwheat. However, there is a definite loss from prolonged heating; heating a concentrated extract for twelve hours resulted in a 14% loss of crude rutin.

REFINING CRUDE RUTIN

Instructions for refining crude rutin have been presented in some detail elsewhere (3, 4). Table VII gives representative data illustrating the purification of rutin to pharmaceutical quality. Samples of crude rutin prepared by the various methods of extraction were purified. The data in Table VII and the spectrophotometric curves in Fig. 1 (9) show that there was little if any difference in the quality of the various crude rutin samples, inasmuch as they " yielded rutin of comparable quality when refined the same process. The refining procedure may be outlined as follows:

A sample of dry rutin (dried to constant weight at 110°), weighing 10.0 Gm., was dissolved in 2 L. of boiling water. The boiling solution was filtered through a heavy filter sheet (K-5), and the filtrate was reheated to boiling. To the boiling solution were added, with continuous stirring, 5.0 Gm. of silica gel (28–200 mesh), and the boiling was continued for five minutes. The solution was filtered through a heavy filter sheet (S-3 of Republic Seitz Corp.) backed with canvas. The rutin solution was cooled and held at a low temperature (5° to 10°) for one hour. The refined rutin was then filtered on hard-surface paper (preferably rayon) and dried to constant weight at 110°. This technique is equally applicable to the purification of large quantities of rutin, as shown in Experiment 166-46 of Table VII.

The quantity of rutin which could be recovered from the mother liquor after recrystallization of the crude product from hot water was determined in Experiment 166-46. The 45 gallons were concentrated to about 2 to 3 gallons and cooled, and the *p*H was adjusted with sulfuric acid to 2.5. The cold solution was allowed to stand for two hours, and the crude rutin was filtered off. The dry weight of this crude material was 22.0 Gm. After two recrystallizations, required to refine it to pharmaceutical quality, 8.4 Gm. of rutin were obtained.

SUMMARY

Hot and cold organic-solvent methods for production of rutin from either green buckwheat plant or dried buckwheat meal are described. The efficiency of various solvents was evaluated, and the best solvent strength and extraction time for optimum yields of rutin were determined. Extraction for ten minutes with hot 85% isopropyl alcohol is recommended for the green plant, since cold solvents give lower yields. Buckwheat meal may be extracted efficiently with either hot or cold solvent, although the hot solvent materially reduces the processing time; 70% to 85% isopropyl alcohol is preferred.

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